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Comprehensive characterization of *Escherichia coli* isolated from urine samples of hospitalized patients in Rio de Janeiro, Brazil

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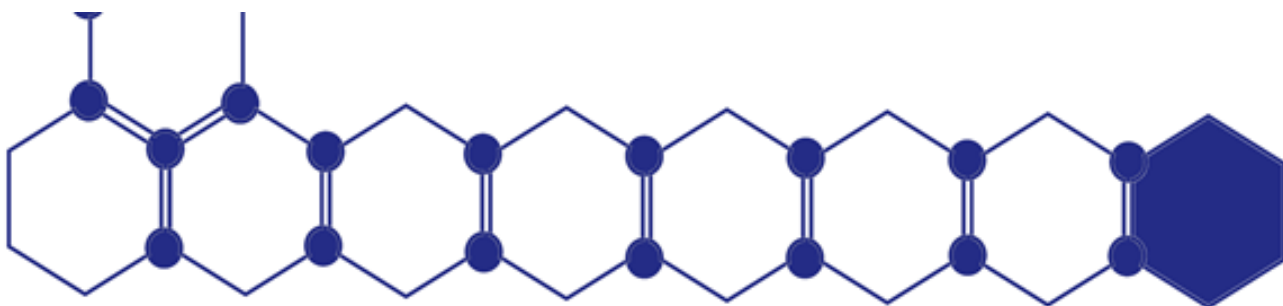
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CHAPTER

1

GENERAL INTRODUCTION AND
RESEARCH QUESTIONS AND
SCOPE OF THE THESIS



General Introduction

Urinary tract Infections

Urinary tract infections (UTIs) are among the most common bacterial infections in both hospitals and the community, affecting around 150 million people worldwide [1], [2]. Age-specific sex-related differences in infections rates are observed. The incidence of UTIs in men until 65 years of age is low and often associated with abnormalities in the urinary tract or with prostate diseases. In contrast, in women the frequency of UTIs is high and it is estimated that one in three women will have at least one UTI episode during their lifetime [2]–[4].

UTIs are classified based on the anatomical site of infections, the patient's underlying risk factors, the severity grade, and microbiological findings. Classification is important for defining an accurate (potential) antibiotic therapy and to avoid the risks of additional complications [5]–[8].

Anatomical classification divides UTIs into two groups. The first group are infections of the lower urinary tract, bladder (cystitis) and urethra (urethritis). The second group consists of infections of the upper urinary tract, particularly the kidneys (pyelonephritis) (Figure 1) [8]. The symptoms of lower and upper UTIs are different. While patients suffering with cystitis often present dysuria, voiding urgency, nocturia, suprapubic pain and hematuria, patients suffering with pyelonephritis present fever, backache and nausea [9]. Additionally, the prostate (prostatitis) or epididymis (epididymitis) may be affected in some cases of lower UTIs in men [10].

UTIs can also be divided into uncomplicated and complicated infections based on the patient's underlying disease and other risk factors present. Uncomplicated UTIs are those occurring in otherwise healthy individuals. UTIs are by definition complicated if the following underlying diseases or risk factors are present: (i) a urinary tract obstruction, (ii) a polycystic kidney, (iii) diabetes, (iv) pregnancy, (v) a neurologic bladder, (vi) presence of an indwelling urethral catheter stent or nephrostomy tube, (vii) a renal transplant (viii), immunosuppression caused by diseases or medicines [5], [9]. In addition, UTIs in male patients and infections caused by multidrug resistant bacteria are also classified as complicated UTIs.

Complicated UTIs can lead to more severe infections such as febrile UTIs and urosepsis and are more difficult to treat. In general, uncomplicated UTIs, mostly community-acquired (CA), are self-limiting and will only be treated with antimicrobial therapy if lasting longer than three days. Hospital-acquired (HA) or nosocomial UTIs are often complicated and it is estimated that 80% is related to indwelling catheters [11][12]. During long catheterization, bacteria form a biofilm in the catheter, facilitating its entrance into the lower urinary tract and enhancing dissemination to the upper urinary tract where they are more difficult to treat [13].

Overall, catheter-associated UTIs (CAUTIs) are associated with increased morbidity and mortality and are often caused by multidrug resistant bacteria [12], [14], [15]. Other risk factors associated with HA-UTIs include surgery (of the urinary tract), patients suffering from diabetes and other comorbidities, and previous admission to a hospital, i.e., between six months and 14 days prior to the current admission [16], [17]. HA-UTIs represent approximately 35-45% of nosocomial infections and lead to an increase in the morbidity, mortality and emotional suffering of hospitalized patients [16].

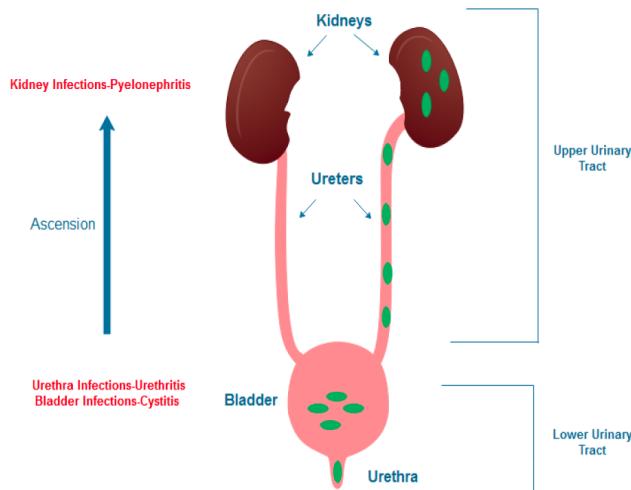


Figure 1. Locations of human urinary tract infections. Infections affecting the upper urinary tract (kidneys) are classified as pyelonephritis and infections affecting the lower urinary tract (urethra and bladder) are classified as urethritis and cystitis. The green circles represent the bacteria in the different locations affected during bacterial UTIs.

Symptoms of UTIs vary from asymptomatic bacteriuria, the presence of bacteria in the urine in the absence of symptoms, to severe urosepsis. Diagnosis is done through physical examination and by using laboratory tests as urinalysis, a group of physical, chemical, and microscopic tests that detect and/or measure several substances in the urine, such as products of normal and abnormal metabolism, cells, cellular fragments, and bacteria. About 50% of women suffering from UTIs present with clinical symptoms and are, therefore, being easier diagnosed. The gold standard for the diagnosis of UTIs is the detection and identification of pathogens through urine culture, with 10^5 colony forming units (cfu)/mL as threshold [18]. However, in patient groups with a higher risk of developing UTIs (e.g., pregnant women and catheterized patients) the clinically relevant threshold is 10^3 cfu/mL for acute uncomplicated cystitis and 10^4 cfu/mL for acute uncomplicated pyelonephritis in women [4], [15].

Treatment with antibiotics is not recommended for asymptomatic UTIs. If treatment is required, however, fosfomycin, nitrofurantoin and trimethoprim-sulfamethoxazole are the first-choice antibiotics for adult patients, using ciprofloxacin, levofloxacin, norfloxacin and ofloxacin as alternatives [21], [22]. In addition, beta-lactams including amoxicillin-clavulanate can be used to treat acute uncomplicated cystitis and fluoroquinolones and beta-lactams are recommended for pyelonephritis [6], [22]. The choice of antimicrobial therapy further depends on the presentation of infections, specific patient groups and the etiological agent causing the infection [19].

Extra intestinal pathogenic *Escherichia coli* as common etioloical agent of UTIs

Escherichia coli are known as part of the normal microbiota of the human gastrointestinal tract, however there are also pathogenic strains. Pathogenic *E. coli* can be divided into two different groups: intestinal (InPEC) and extraintestinal *E. coli* (ExPEC) [20]. UTIs can be caused by several microorganisms, *E. coli* being the most common etiological agent [21], [22] causing around 80% of UTIs. Particularly, ExPEC can colonize different parts of the human body and have, compared to commensal *E. coli*, a bigger genome with a higher number of virulence genes, mainly adhesins, invasins, fimbriae, toxins and genes involved in the iron uptake systems. The presence of these virulence factors is essential for both ExPEC's ability to survive in the gastrointestinal tract of healthy individuals and for causing infections in other body sites [23], [24].

The ability of ExPEC to survive and colonize in the gastrointestinal tract is an important factor in the pathogenesis of UTIs. It is believed that most UTI-causing strains originate from the host fecal flora [25] due to the short proximity between urethra and anus in

female patients. This also explains the higher incidence of UTIs among women [26].

The pathogenesis of *E. coli* causing a UTI exists of the following steps: (i) urethra colonization, (ii) bladder ascension and growth of planktonic cells in urine, (iii) adherence to the surface and interaction with bladder epithelium. After these three steps, *E. coli* can form biofilms and/or invade epithelial cells and replicate herein forming bladder intracellular bacterial communities (IBCs) or quiescent intracellular reservoirs where they can persist being protected against the host-defense mechanisms [57]. These reservoirs are therefore associated with recurrent and chronic UTIs caused by the resurgence of the intracellular pathogens [28], [45]. In addition, by invading the urothelial cells the uropathogens can gain access to additional nutrients and be more easily translocated by host cells. Subsequently, kidney colonization and host tissue damage may occur, increasing the risk of bacteremia/septicemia [26].

The severity of UTIs depends on both host factors and the presence of specific virulence factors in the *E. coli* bacterium. The human host has several defense mechanisms such as the iron limitation in the urine, specific antibodies, or presence of antimicrobial peptides to prevent the colonization and infection caused by microorganisms [27]. *E. coli* causing UTIs contain specific virulence factors to overcome the host's defense strategies (Figure 2). Among ExPEC associated with UTIs a specific group, named uropathogenic *E. coli* (UPEC) has been defined. UPEC contain two types of virulence factors. The first type consists of virulence factors located on the bacterial cell surface as, e.g., outer membrane proteins, including fimbria, pili, curli and adhesins, critical for binding to the cells of the urinary tract [28, 56]. Type 1 fimbriae that bind urothelial mannosylated glycoproteins are known to be associated with the enhanced survival of *E. coli* in the urinary tract and are also involved in invasion of urothelial cells. The presence of P fimbriae is associated with ascension of UPEC isolates leading to the development of pyelonephritis [29]. Other fimbriae such as S fimbriae and F1C interact with the host cells present in the low urinary tract and kidneys [29]–[31]. Finally, the iron uptake systems such as aerobactin or siderophore are essential to overcome the iron limitation in the host environment such as urine [32].

The second group of virulence factors consists of secreted proteins such as toxins and lipoproteins that can lyse host cells, allowing UPEC to easier cross mucosal barriers, damage effector immune cells and access host nutrients and iron stores [29]. Toxins secreted by UPEC such as the alfa-hemolysin (HlyA), cytotoxic necrotizing factor type 1 (CNF1) and secreted autotransporter toxin (SAT) play an important role in the colonization of the urinary tract. The alfa-hemolysin (HlyA) is a pore-forming toxin that affects mainly erythrocytes and contributes to nephropathogenicity, while the CNF-1 interferes with polymorphonuclear phagocytosis and death of bladder epithelial cells. SAT is associated with pyelonephritis and toxicity against urinary tract cells [33], [34].

Among the secreted virulence factors, bacteriocins, antibacterial peptides that kill closely related bacteria [35], [36], are well-known. Usually multiple, different bacteriocins are being produced [35]. They may help UPEC to outcompete other bacteria in an environment with limited nutrients and therefore increase its virulence potential [35], [37]. Colicins and microcins are the bacteriocins mostly associated with UPECs. Colicins can be classified into group A and group B. The group A colicins are translocated by the Tol system and group B colicins use the TonB system. In addition, group A colicins are normally encoded by small plasmids and released into the medium, while group B colicins are encoded by large plasmids and are not secreted [38], [39]. Colicins present different action mechanisms as, e.g., disrupting the bacterial membrane by forming a pore, interrupting macromolecular synthesis, or damaging bacterial DNA through their nuclease activity [38], [40].

Microcins are low molecular weight peptides produced by UPEC and some are related to siderophores [41]. Different from colicins, microcins are post-translationally modified and are not induced by the SOS system, responsible for a global response to DNA damage in which the cell cycle is arrested and DNA repair and mutagenesis is induced [42]. Microcin V has been frequently identified in UPEC causing pyelonephritis [35]. This microcin is encoded by a heterogeneous group of large virulence related plasmids, that was also identified among avian pathogenic *E. coli* strains [41]. Microcins can be classified into Class I microcins that include the peptides with a molecular mass below 5 kDa, e.g. B17 and C7, and Class II microcins ranging from 5 to 10 kDa. Class II microcins can be further subdivided into IIa, those containing disulfide bonds, e.g. V and IIb, and linear microcins, e.g. H47 and M, that carry a C-terminal posttranslational modification containing a salmochelin-like siderophore moiety [43]. Interestingly, the microcin V and M, from the IIa and IIb class, respectively, are phylogenetically associated [41].

Bacteriocin production is widely distributed in nature and is particularly observed in the gut of animals. Its production is stimulated by several factors, including the stringent response, catabolite repression, mutations in specific genes, e.g. the *ompR* gene, stationary phase of the growth, anaerobiosis, high temperature and nutrient depletion [38].

Overall, the production of bacteriocins are associated with *E. coli* strains belonging to phylogenetic group B2 present in human fecal samples. Interestingly, most ExPEC isolates belong to this phylogenetic group [44]. Overall the profile of the virulence factors in UPEC isolates is highly diverse and, for this reason, establishing a relation between the presence of virulence factors and severity of the infections is difficult. Still, identifying virulence factors could be used to search for molecular markers for more accurate diagnostics and as potential vaccine targets [45].

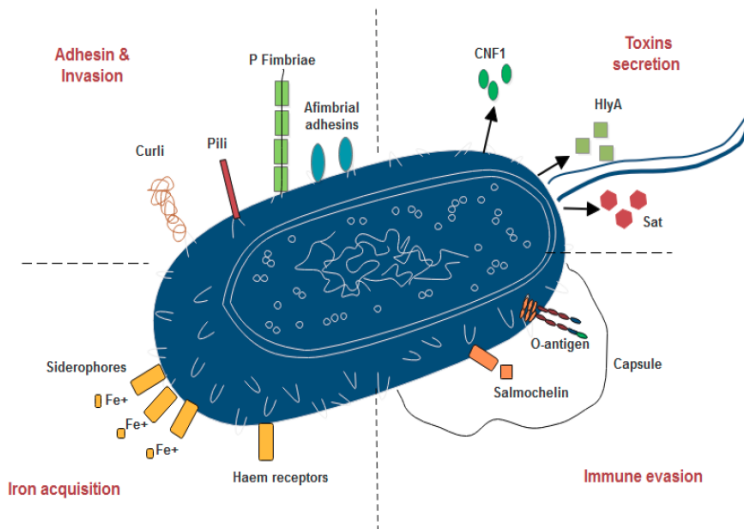


Figure 2. Virulence factors of uropathogenic *Escherichia coli*. The virulence factors can be divided into four main groups: adhesins and fimbriae, both associated with the adhesion of the bacteria to host urothelial cells; iron acquisition proteins, essential to survive in and to colonize the urinary tract in an iron limited environment; and toxins, associated with immune evasion, exfoliation and tissue damage.

Crucial for the persistence of *E. coli* in the genitourinary tract is the ability to form a biofilm [46] and it is estimated that 68-80% of *E. coli* can do so [47]–[49]. Bacterial cells present in biofilm are known to have increased resistance to antibiotics, detergents and host immune defense substances [46], [50]. UPEC can form an intracellular biofilm community (IBC) in urogenital cells and a biofilm on abiotic surfaces of indwelling medical devices, including urinary catheters [51]. Therefore, CAUTIs are one of the most common nosocomial infections [13], [52]. *E. coli* can also form biofilms into the bladder walls[51]. Biofilm formation hinders the treatment of UTIs, as they “protect” the bacteria against the antibiotic therapy, and is, therefore, also associated with chronic UTIs, such as chronic prostatitis [51], [53]. Indeed, prolonged catheterization can result in persistent infections and inevitable lead to bacteriuria. Catheters also impair the normal defense of the bladder, further increasing bacterial colonization. Moreover, bacteria in biofilms work as a community, facilitating the exchange of genetic material thereby facilitating the spread of antibiotic resistance. Finally, biofilms complicate an accurate diagnosis and antibiotic susceptibility testing as only the bacteria floating in the urine will be collected. The most effective way to avoid CAUTIs is to prevent catheterization or reduce the duration of it [22], [54]. This also reduces the reservoir of MDR gram-negative bacteria inside hospitals [55].

Mobile genetic elements

Many of the virulence factors are encoded by genes on mobile genetic elements (MGEs), especially pathogenicity islands (PAIs). PAIs are a distinct class of genomic islands that are usually absent from nonpathogenic microorganisms. They are horizontally acquired and can originate from non- related microorganisms. PAIs are considered to be involved in ExPEC’s pathogenicity and play a role in the evolution of hypervirulent *E. coli* clones [58], [59]. Virulence genes present in PAIs include adhesins, toxins, invasins, capsule genes, and genes belonging to the iron uptake system and secretion system [61]. The PAIs are characterized by tRNA genes, GC-content, repeated sequences and insertion sequences (ISs) [58], [62]. UPECs can have several PAIs and there is an association between the presence of PAIs and the severity of infections [63].

Also, plasmids containing genes encoding bacteriocin, toxins, genes belonging to the iron uptake systems and outer membrane protein genes, have been associated with virulence in ExPEC, particularly extended-spectrum-beta-lactamase(ESBL)-encoding plasmids belonging to the IncF, A/C, N, and K types[66]. The acquisition of plasmids is further associated with bacteria causing outbreaks and with emergence of new resistance clones [60], [67].

Finally, other MGEs as transposable elements and bacteriophages may contain virulence genes contributing to the virulence of ExPEC [60].

Antibiotic resistance and *E. coli* high-risk clones

In the last years, the number of infections caused by multidrug-resistant (MDR) *E. coli* has increased worldwide. Commonly used antibiotics as fluoroquinolones and cephalosporins to treat UTIs cannot be used against such resistant bacteria, necessitating the use of last-resort antibiotics as carbapenems for which also increasing resistant rates are reported [1]. Indeed, UTIs with ESBL- producing and fluoroquinolones resistant *E. coli* are of big concern [68] and empirical treatment of UTIs is challenging [69]. Among the ESBL-producing *E. coli* in Europe and the Americas, 97% contains a class A beta-lactamase, CTX-M gene [70], [71]. The spread of these resistance genes, especially that of the CTX-M-15 gene, is being observed since early 2000s and is associated with the emergence of specific *E. coli* lineages. It is strongly suggestive that the emergence, expansion and rapid dissemination of specific lineages with specific antibiotic resistance gene profiles are associated with particular geographical regions related to the use of specific antibiotic groups in that regions [72]–[74].

Isolates belonging to successful lineages are also called high-risk clones and often display a MDR- phenotype with increased levels of virulence. Such high-risk clones are highly abundant in the community and in the hospital environment. One of the most often studied high-risk clone of *E. coli* is the ST131 lineage, which became a worldwide dominant clone since the beginning of the third millennium [75], [76]. The successful expansion of one of the sub-clones of this lineage was shown to be driven by the presence of the type 1 fimbriae encoded by the *fimH30* gene (ST131 sub-clone *H30*). Especially, a specific subgroup, assigned as *H30-Rx*, mostly associated with isolates resistant to fluoroquinolones and cephalosporins and producing CTX-M-15, rapidly spread worldwide in the recent years [77]. By analyzing the presence of specific virulence genes, the ST131 *E. coli* can be divided into virotypes A, B, C and D, with virotype C being the most prevalent showing a worldwide dissemination [77], [78]. The other virotypes are more restricted to specific regions [79].

Next to ST131, other lineages as ST69, ST73, ST405 and ST648 successfully expanded worldwide [80]. Most ST69 and ST73 isolates are normally not associated with ESBL production [81]. However, recent studies reported ESBL-producing isolates belonging to these lineages, stressing the importance of monitoring the emergence of new ESBL-producing clones [82]. In addition, ST69 has been associated with the increase of resistance to trimethoprim in different countries including Brazil. MGEs already discussed in these chapter to be important for acquisition of genes encoding virulence factors also play an important role in the spread of resistance genes [67]. Especially, plasmids belonging to incompatibility group IncF are known to often carry resistance genes and are disseminated among the whole family of Enterobacteriaceae. These plasmids contain divergent replicon types such as FIA, FIB and FII, and have the ability to carry resistance cassettes [83]. Overall, plasmid mediated horizontal gene transfer (HGT) is considered to be the most important mechanism for spread of antibiotic resistance [84].

In Brazil, antibiotics are available even without prescription, despite laws to control and reduce the abusive use of it. The combination of the abusive antibiotic use and the spread of highly virulent and resistant clones is even enhancing the antibiotic resistance crises. It is estimated that already 700,000 people per annum die of infections that cannot be treated at all [68]. Furthermore, a better choice of current antimicrobial agents, improved diagnostic methods (including the detection of these high-risk clones), infection prevention measures, and extensive surveillance are extremely important and required to prevent further spread of antibiotic resistance.

Fosfomycin (hetero)resistance

With the increase of antibiotic resistance, old drugs, including fosfomycin have been studied and used to treat infections caused by thrimetoprim-sulfametazaxole resistant and ESBL- and carbapenemase-producing bacteria [85]. Fosfomycin was discovered in 1969 in Spain and is active against both Gram-positive and -negative bacteria. Although, it has low resistance rates (1- 5%) and works against biofilms, the drug was not used for a while mainly due to the poor bioavailability of oral fosfomycin (37%) and its limited effectiveness in patients who are critically ill [86]. Additionally, side effects have been reported such as angioedema, aplastic anemia, cholestatic jaundice and hepatic necrosis [86]. Despite the presence of side effects, fosfomycin has been used to treat uncomplicated UTIs and recent studies indicated it to be a good choice for treating UTIs caused by MDR *E. coli* [87]–[89].

Fosfomycin acts by inhibiting the first cytoplasmic stage of cell wall synthesis by binding to the enzyme UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) thereby inhibiting its activation. To enter the bacteria, fosfomycin uses two different transporters, L-alpha-glycerol-3-phosphate (GlpT) and hexose-6-phosphate (UhpT) [85]. The activity of the second transporter is induced by the presence of glucose-6-phosphate. In addition, expression of both transporters is regulated by cyclic AMP (cAMP) [90]. Furthermore, fosfomycin can reduce the adhesion of the bacteria to the epithelium [91] (Figure 3). Although both the mechanism of action of fosfomycin and its structure are unique, making cross-resistance unusual, resistance to fosfomycin can occur through several other mechanisms. The best known is a mutation in the *murA* gene resulting in an amino acid change of a cysteine into an aspartate found in several bacteria like *Vibrio ischeri*, *Chlamydia spp.* and *Mycobacterium tuberculosis* [91] and due to which fosfomycin cannot bind to MurA any longer. Another resistance mechanism is the production of peptidoglycan via an alternative route, as has been identified in *Pseudomonas putida* (recycling route) [92].

In *E. coli*, fosfomycin resistance has been described to be caused by mutations in the chromosomally located *glpT* and *uhpT* genes encoding fosfomycin transporters resulting in blocking the uptake of fosfomycin [93]. Since these proteins are also essential for bacterial survival and their metabolism, such mutations are rarely found. More commonly found mutations include those in the *gyaA* and *ptsI* genes, resulting in lower cAMP levels thereby decreasing the expression of fosfomycin transporters. In addition, the overexpression of MurA is also related to fosfomycin resistance in *E. coli* [93], [94].

Enzymes capable of modifying fosfomycin, such as glutathione S-transferase (FosA), a metalloenzyme transferred through plasmids among Enterobacteriaceae, can also cause fosfomycin resistance. Several subtypes of glutathione S-transferases with similar structures have been described (FosA2, FosA3, FosA4 and FosA5) [95], [96]. Other enzymes involved in fosfomycin resistances include FosB, an enzyme 46% identical to FosA and catalyzing a reaction between cysteine and fosfomycin in Gram-positive bacteria, FosX, a chromosomal enzyme catalyzing a reaction of fosfomycin with water and found in *Listeria monocytogenes*, and FosC, an enzyme similar to glutathione S-transferase catalyzing the phosphorylation of ATP and inactivating fosfomycin found in *Pseudomonas syringae* [97]–[99].

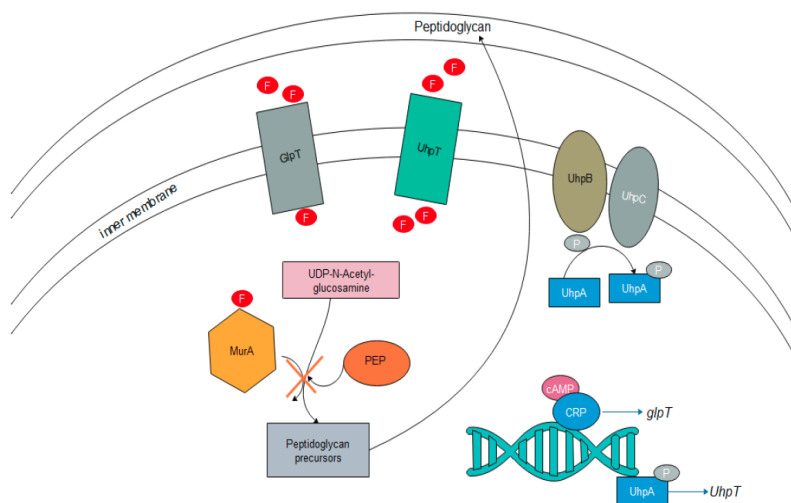


Figure 3. Fosfomycin uptake and regulation of transporters. Fosfomycin can be transported by both GlpT and UhpT. Once inside the cell, fosfomycin binds to MurA blocking the catalysis and, consequently, the formation of UDP-GlcNac-3-O-enoulypyuvate a peptidoglycan precursor using UDP-GlcNac and PEP. The expression of the *glpT* and *uhpT* genes are induced by the cAMP-CRP complex and UhpA.

Even though the fosfomycin resistance rate remains low, heteroresistance to fosfomycin may be an underestimated problem. The precise definition of heteroresistance is not fully clear and standardized. The heterogeneity inside a bacterial population is relatively common, and can generate subpopulations that tolerate high concentrations of antibiotics but that are not detected in routine diagnostics [100]. However, different from such tolerant subpopulations, heteroresistant strains can be defined as the presence of one or more subpopulations in a particular bacterial population that is/are able to grow in the presence of concentrations of the antibiotic higher than their minimum inhibitory concentration (MIC) [100]. In addition, a heteroresistant profile is normally not reversible [101]. Heteroresistance can lead to treatment failure [100], [102], and may be particularly relevant for UTIs where bacteria form IBCs. Once fosfomycin penetrates cells, it normally kills the bacteria in these IBCs present inside the urothelial cells. However, due to the heteroresistance phenotype, resistant subpopulations survive and may cause persistent and recurrent UTIs [103].

Heteroresistance to fosfomycin has been described for *S. pneumoniae*, *P. aeruginosa* and *E. coli* [89], [94], [97][90], [102]. Previous studies have linked this phenotype to mutations in genes that regulate cAMP, *ptsI* and *cyaA* genes, in genes encoding transporters used by fosfomycin, i.e., *glpT* and *uhpT*, in genes that regulate the expression of these transporters such as *glpR*, *uhpA*, *uhpB*, *uhpC*, resulting in reduced expression of the transporters used by fosfomycin and consequently its uptake, and mutations in the fosfomycin target *murA* gene, preventing the binding of fosfomycin to its active site. However, the few studies addressing these mechanisms state that such mutations come with a high cost of bacterial fitness being the reason that the frequency of heteroresistance is even lower than full resistance [90], [102], [104]–[106]. Clearly, studies evaluating the clinical impact of heteroresistance against fosfomycin and the mechanisms leading to this phenotype are highly relevant for a better understanding of the importance of this profile in UTIs and other kind of infections.

Research questions and scope of the thesis

In general, infections inside hospitals are considered a public health problem in Brazil leading to an increase in morbidity and mortality as well as an increase in health care costs. Particularly, UTIs are one of the most common HA-infections. MDR-bacteria as *E. coli* have led to serious and difficult-to-treat infections. There is a need to improve the management of UTIs in patients, especially in cases where infections are caused by MDR *E. coli*. This can only be achieved by improving diagnostic methods and the most prudent use of currently available antibiotics. Thus, new, rapid molecular diagnostic techniques and better understanding the mechanisms of virulence, resistance and dissemination of rapidly emerging strains will be of direct benefit for the patient. The use of whole genome sequencing (WGS) that provides insights into the virulence and resistance mechanisms of bacterial pathogens, can be one of these strategies. Through WGS we can assess the risk of emerging pathogens, predict epidemiological patterns, comprehensively understand the evolution and transmission of high-risk bacterial clones and understand their epidemiology. This knowledge is key for improvement of treatment and infection prevention strategies.

As mentioned, *E. coli* is the most common etiological agent of UTIs and increased resistance rates to antibiotics normally used to treat these infections are observed. Although an association between specific sequence types and the resistance profile has been found in several countries, there is a lack of information for South American countries, particularly Brazil. Therefore, getting insight into the local epidemiology of *E. coli* in these regions is key for developing infection prevention strategies.

Thus, our initial research questions were: How is the population structure of these isolates in Brazil? What is the frequency of antibiotic resistance? and Could resistance be associated with specific lineages?

These questions were addressed in chapter 2 of this thesis which had as main objective to comprehensively characterize the population structure of *E. coli* from urine samples collected from patients in four hospitals in Rio de Janeiro, Brazil using WGS.

MGEs play an important role in the virulence and resistance of *E. coli* and are known to drive the evolution of specific successful lineages spread worldwide. Among MGEs, genomic islands, particularly PAIs, horizontally transferred between bacterial cells, can dramatically increase the virulence genes content allowing these bacteria to overcome host defense mechanisms and to cause more severe infections. In addition, the acquisition of plasmids is known as the main mechanism to acquire antibiotic resistance and is associated with the emergence of high-risk *E. coli* clones. Furthermore, plasmids are known to drive the evolution towards specific sub-clones of *E. coli* that are more virulent and associated with complicated UTIs.

Therefore, our next questions were: Which MGEs can be identified in our *E. coli* isolates? What is the role of these MGEs in the virulence and resistance profiles of these isolates? And Is there an association between plasmids and specific sub-clones of ST131 isolates?

We tried to answer these questions in chapter 3 of this thesis which had as main objectives to characterize the MGEs present in ST131 *E. coli* isolates and other lineages isolated from urine of hospitalized patients, in Rio de Janeiro, Brazil and to reveal the role of MGEs in the antimicrobial resistance and virulence of the isolates.

As the treatment of UTIs caused by multidrug-resistant *E. coli* is at least challenging, old antibiotics, such as fosfomycin have been studied as an alternative option to treat complicated UTIs caused by MDR bacteria. Previous studies showed a high effectiveness of fosfomycin against this pathogen with low resistance rates. Despite the resistance rate of fosfomycin remaining low, another phenomenon could lead to reduced susceptibility to this antibiotic, i.e., heteroresistance against it, that, as mentioned, is a poorly investigated topic. In heteroresistant populations, subpopulations able to growth in the presence of high concentrations of antibiotics can lead to therapy failure and could be associated with recurrent infections. However, there are only few studies about fosfomycin heteroresistance and its definition and methodologies to study it are not standardized.

Therefore, the next questions addressed in this thesis were: What is the frequency of fosfomycin heteroresistance among our multidrug resistant *E. coli* isolates? What are the molecular mechanisms associated with this phenotype? and Can a heteroresistance profile affect the susceptibility of bacterial cells to antibiotics in biofilms??

We addressed these questions in chapter 4 of this thesis which had as main objectives to study the frequency of heteroresistance in *E. coli* isolated from urine of hospitalized patients in Brazil, to characterize the possible molecular mechanisms involved in it and to investigate the potential effect of the heteroresistance in the eradication of (bacteria in) biofilms.

As mentioned, virulence factors are essential for *E. coli* pathogenesis. Bacteriocins are a group of antibacterial peptides produced by bacteria that allow them to kill other closely related bacteria. They are one of the virulence factors that can increase the survival of bacteria by increasing their competitiveness. Bacteriocins are frequently encoded by plasmids and are produced by both commensal and pathogenic *E. coli* including those present in the human gut microbiota. Bacteriocins could help specific lineages to colonize the human gut making it a reservoir for these pathogens that then can cause UTIs by contaminating the urinary tract. However, the number and types of bacteriocins present in *E. coli* isolates is extremely diverse and until now no indication of specific bacteriocin genes associated with resistant lineages was found. Nonetheless, the presence of bacteriocin and resistance genes on the same plasmid can enhance the spread of specific bacteriocin genes among lineages associated with antibiotic resistance. Hence, the questions addressed in this chapter were: Could the presence of plasmids encoding bacteriocin and resistance genes affect the virulence potential of bacteria? and Could the presence of resistance genes in bacteriocin encoding plasmids enhance transmission of this plasmid?

These questions were addressed in chapter 5 of this thesis which had as main objectives to characterize resistance genes and colicin-encoding plasmids of ST131 *E. coli* isolated from clinical urine samples and to investigate their role in the virulence of these isolates.

Virulence factors such as adhesins, invasins, iron uptake systems and biofilm-forming ability are essential in the pathogenesis of *E. coli* as they allow the bacteria to colonize and survive in the host. The biofilm-forming ability is associated with chronic and device-related UTIs. However, the presence of resistance and virulence determinants in *E. coli* isolates represent only half of the factors that contribute to the risk of developing a UTI; the other half is related to the host's susceptibility. The presence of several risk factors is

known to increase the host susceptibility and the risk of therapeutic failure. Despite several studies have been performed to better understand the relation between the genetic characterization of pathogens and the risk factors in patients suffering from UTIs, the genotypic diversity among ExPEC strains makes it difficult to find such associations and more studies are required to get a better understanding of it.

Therefore, our next questions were: Could we correlate the biofilm-forming ability with virulence and resistance phenotypes and genotypes? and Is there an association between the pathogenicity of the bacteria and the presence of risk factors of the patients?

These questions were addressed in chapter 6 of this thesis which had as main objective to reveal characteristics of UTIs in hospitalized patients in Rio de Janeiro, Brazil, including the biofilm-forming ability and antibiotic susceptibility of the bacteria, the presence of bacterial virulence and resistance genes, and a possible correlation between the patient's risk factors and getting a CA-UTI or HA-UTI.

Finally, in the chapter 7 of this thesis we discuss our findings, try to make conclusions out of them and give some future perspectives.

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